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Ginger L. Chew[1], Jonathan Wilson[2], Felicia A. Rabito[3], Faye Grimsley[4], Shahed Iqbal[3], Tiina Reponen[5], Michael L. Muilenberg[6], Peter S. Thorne[7], Dorr G. Dearborn[8], and Rebecca L. Morley[2]

1 Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY

2 National Center for Healthy Housing, Columbia, MD

3 Department of Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

4 Department of Environmental Health, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

5 Department of Environmental Health, University of Cincinnati, Cincinnati, OH

6 Department of Environmental Health, Harvard School of Public Health, Boston, MA

7 Department of Occupational & Environmental Health, The University of Iowa, College of Public Health, Iowa City, IA

8 Department of Environmental Health Sciences, Case Western Reserve University, Cleveland, OH

Correspondence and requests for reprints should be addressed to: Dr. Ginger L. Chew, The Mailman School of Public Health, Columbia University, 60 Haven Avenue, B-1, New York, New York 10032-4206. Telephone # (212) 305-1692, Fax # (212) 305-4012, e-mail : cg288@columbia.edu.

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Abbreviations and Acronym List:
APF = Assigned protection factor
ARC = American Red Cross
CFU= Colony forming unit
DG-18 = Dichloran glycerol
EU = Endotoxin units
GM = Geometric mean
GNOCDC = Greater New Orleans Community Data Center
GSD = Geometric standard deviation
HEPA = High efficiency particulate air filter
HVAC = Heating ventilation/air conditioning
LSU = Louisiana State University
MEA = Malt extract agar
NCHH = National Center for Healthy Housing
NHS = Neighborhood Housing Services of New Orleans
NIEHS = National Institute of Environmental Health Sciences
NIH = National Institutes of Health
NORA = National Occupational Research Agenda
NYCDOHMH = The New York City Department of Health and Mental Hygiene
PCR = Polymerase Chain Reaction
qPCR = quantitative Polymerase Chain Reaction
URC = Urban Renovation Consultants
USDA = United States Department of Agriculture
U.S.CDC = United States Centers for Disease Control and Prevention
U.S.EPA = United States Environmental Protection Agency
U.S.OSHA = United States Occupational Safety and Health Administration
WPF = Workplace Protection Factor

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Abstract

BACKGROUND: Following Hurricane Katrina, many New Orleans homes remained flooded for weeks, promoting heavy microbial growth.

OBJECTIVES: A small demonstration project was conducted November 2005-January 2006 with the aims to recommend safe remediation techniques, safe levels of worker protection, and to characterize airborne mold and endotoxin throughout clean-up.

METHODS: Three houses with floodwater lines between 0.3 and 1.8 m underwent intervention including disposal of damaged furnishings and drywall, cleaning surfaces, drying remaining structure, and treatment with a biostatic agent. We measured indoor and outdoor bioaerosols before, during, and after intervention. Samples were analyzed for fungi (culture, spore analysis, polymerase chain reaction (PCR)) and endotoxin. In one house, real-time particle counts were also assessed and respirator efficiency testing was performed to establish Workplace Protection Factors (WPF).

RESULTS: At baseline, culturable mold ranged from 0.22-5.2x10^5 colony-forming units/m^3, spore counts ranged from 0.82-6.3x10^5 spores/m^3, and endotoxin ranged from 17-139 endotoxin units/m^3. Culture, spore analysis, and PCR indicated that *Penicillium, Aspergillus, and Paecilomyces* predominated. After intervention, levels of mold and endotoxin were generally lower (sometimes, orders of magnitude). The average WPF against fungal spores for elastomeric respirators was higher than for the N-95 respirators.

CONCLUSIONS: During baseline and intervention, mold and endotoxin levels were similar to those found in agricultural environments. We strongly recommend that those entering, cleaning, and repairing flood-damaged homes wear respirators at least as protective as elastomeric respirators. Recommendations based on this demonstration will benefit those involved in the current clean-up activities and will inform efforts to respond to future disasters.

Introduction

Hurricane Katrina, which occurred on August 29, 2005, caused the breach of several levees surrounding the City of New Orleans and left over 75% of New Orleans under water. A storm surge from Hurricane Rita several weeks later re-flooded many areas of the city. In the aftermath of these disasters, homes were submerged in flood water for several summer weeks, resulting in severe mold and bacteria growth, both of which can lead to a panoply of respiratory health effects (Institute of Medicine 2004). While several studies have assessed markers of mold and bacteria in damp and water-damaged homes (Institute of Medicine 2004), there is a dearth of literature documenting the levels of these agents in homes that received sustained flooding (Ross et al. 2000).

New Orleans and the surrounding communities currently face difficult decisions regarding whether or not to demolish large numbers of damaged homes. Many factors will be taken into consideration when those decisions are made, including the question of whether mold and bacteria can be safely and affordably removed from the flooded homes prior to building restoration. On October 27, 2005, the National Center for Healthy Housing (NCHH) convened a meeting to examine options for flood clean-up procedures and air sampling. Attendees included building materials scientists, mold remediation experts, members of community-based organizations, industrial hygienists, clinicians, housing policy makers, and environmental epidemiologists from across the country (including areas affected by Hurricane Katrina). The attendees drew on a body of guidance documents for flood cleanup and mold remediation (ARC and FEMA 1992; LSU 2005; NYCDOHMH 2002; U.S.CDC 2005; U.S.EPA 2001).

The overall purpose of this demonstration was to assess the efficacy of flood clean-up procedures in three houses in New Orleans, which sustained between 0.3-1.8 m of flood damage from Hurricanes Katrina and Rita. Three specific objectives of the demonstration were to: 1) make recommendations for safe re-entry into a home and safe removal of flood-damaged furnishings and building materials, including levels of worker protection; 2) characterize the distribution of airborne mold and endotoxin (a cell wall component of Gram negative bacteria) throughout all phases of the clean-up; and 3) comment on the practicality of

the overall clean-up process. To this end, Enterprise Community Partners and NCHH, in collaboration with Neighborhood Housing Services of New Orleans (NHS) and several partners from academic institutions, studied these issues as part of the demonstration project, which was conducted between November 2005 and January 2006.

Materials and Methods

Selection of homes: Three single-family houses in the Gentilly district of New Orleans were selected to participate in the project. Most of the areas in Gentilly lie 0.5 to 4 meters below the sea level (GNOCDC 2006). According to a recent report, 85% of the total households in this area suffered some degree of damage and 71% households were severely damaged or destroyed by flood from Hurricane Katrina (GNOCDC 2006).

To be included in the demonstration, a house had to be: flooded with less than 1.8 m of water, structurally sound, and located in an area likely to be rebuilt. Owners were required to have flood insurance deemed adequate to rebuild their house and intentions to do so. The first house was selected when an employee of New Orleans-based housing non-profit volunteered his house to the project. NHS, a chartered member of NeighborWorks America, identified two additional homeowners whose homes met the inclusion criteria. At House #1, the homeowner selected the contractor to complete the work. At the latter two houses, NHS of New Orleans selected the contractors, and with support from NCHH and Urban Renovation Consultants (URC), supervised the activities. URC, NCHH, and its national advisory work group developed the construction specifications used at the three houses.

Personal Protective Equipment. All workers and supervisors wore N100 filtering facepiece respirators, nonwoven polypropylene disposable coveralls, and gloves during the property inspection. During property removal and deconstruction, some workers additionally used half-face high efficiency particulate air filter (HEPA) air-purifying respirators (some equipped with organic solvent filters) and goggles.

Baseline inspection. The supervisors visually inspected each house for roof leakage, standing water, and the extent of mold on walls, cabinets, floors, doors, trim, appliances, equipment, and heating ventilation/air conditioning (HVAC) ductwork. The moisture content of the wood studs was tested using a moisture meter with a probe (J-LITE Moisture Meter, Delmhorst Instrument Company, Towaco, NJ) (moisture readings > 22% were considered saturated and < 15% were considered dry) (USDA 1999). The supervisors determined whether electrical service, if operable, was safe and verified that there were no water or gas leaks. In House #1, surfaces were relatively dry at the time of the demonstration, with no standing water. Possessions with visual mold and limited value to the owner of House #1 were discarded. Other hard surface possessions were cleaned outside with a bleach-detergent solution and then packed in boxes and moved to the second floor of House #1. House #2 had been closed since Hurricane Katrina and was still relatively wet at the start of the project, with standing water, wet furnishings, and plaster and drywall surfaces saturated with water In House #3, personal belongings, furniture, and lower cabinets had been removed by the time of the initial inspection. The carpet and padding were wet, and the drywall was saturated within 0.3 m of the floor. Details of the baseline conditions and demonstration activities are listed in Table 1.

Deconstruction. Deconstruction at all houses included removing carpet or otherwise clearing floors down to the finished flooring, and removing insulation, nails on studs and lower cabinets, if present. At House #1, bathroom toilets, sinks, and bathtubs were also removed. At House #2, workers also removed upper cabinets.

Cleaning and sanitizing. Workers cleaned and sanitized all three houses with a combination of dry cleaning and wet cleaning steps. Workers conducted dry cleaning by bristle brushing studs and other framing members to remove visible mold growth and then vacuuming the same surfaces with a HEPA

filter attachment. Wet cleaning was completed by using sponge mops and hand sponges to wash down surfaces with a non-tri-sodium phosphate solution of sodium sesquicarbonate detergent and 15% dilution of household chlorine bleach. In all three houses, dry cleaning was conducted before wet cleaning. In House #2, all surfaces including all framing members were wet cleaned. In Houses #1 and 3, workers wet cleaned only hard, nonporous surfaces (e.g., tubs, sinks, toilets, tile flooring).

Biostat treatments. After cleaning, the three houses received biostat treatment with one of two borate formulations to prevent future emergence of mold. House # 1 was treated with Bora-Care (Nisus Corp., Rockford, TN), House #2 was treated with Bora-Care in half the house and Termite Prufe (Copper Brite, Inc., Santa Barbara, CA) in the other half, and House #3 was treated with Termite Prufe. The active ingredient in both Bora-Care and Termite Prufe is disodium octoborate tetrahydrate. Both products are registered fungicides by the US Environmental Protection Agency. Bora-Care was mixed with water (1:3 or 1:4) and sprayed onto all lumber sheathing to the point of wetness in conformance with the manufacturer's instructions. Termite-Prufe (0.45 kg of Termite Prufe powder to 3.8 liters of water) was applied to all exposed interior surfaces at 18.7 m^2 per 3.8 liters, working from ceiling to floor. The two formulations differ significantly in cost, after mixing.

Drying. Windows in Houses #2 and 3 were left open to allow cross-ventilation to dry the moisture introduced by the biostat treatments. All possessions had been removed from these houses, so security was not a concern. In House #1, some possessions were stored on the second floor, thus windows were closed, but the upstairs ceiling fan was utilized. No mechanical dehumidification was used in any house.

Air sampling

Air sampling was conducted during three timepoints for each home: 1) before intervention (i.e., Day 1), 2) during intervention (i.e., Day 1 or 2), and 3) after intervention (i.e., Day 36, 23, and 15, respectively,

for Houses 1, 2, and 3). The pre-intervention sampling for House#1 occurred one day after some items were removed from the house, but before work commenced on the second day. For Houses #2 and #3, the pre-intervention sampling occurred either one day or a few hours before demonstration commenced. Intervention sampling occurred within a few hours of removal of mold-laden drywall. Post intervention sampling occurred between one and two weeks after borate solution was applied. Table 2 summarizes the air sampling activities conducted in each home.

Indoor samples: Using battery-operated air sampling pumps (AirChek 2000, SKC, Inc., Eighty Four, PA), the living room air was sampled with 2.0 μm pore-size Teflon filters (Omega Specialty, Inc., Chelmsford, MA) housed in 37-mm cassettes for 20 min at a flow rate = 2.5 liters per minute (lpm). In some houses, additional samples were collected upstairs (House #1) and in the bedroom (House #3). The pumps were attached to a tripod 1.2-1.35 m above the floor with the sampling cassette open to the center of the room. Using high flow (flow = 15 lpm) air sampling pumps (Environmental Monitoring Systems, Inc., Charleston, SC), fungal spores were collected in the living room air using BioCell™ impaction cassettes (GrafTech, Inc., Brighton, MI) for 2 min pre-and post-intervention, and 1 min, during intervention. Sampling trains were pre- and post-calibrated, and the average flow rate was used in calculations of volume of air sampled.

Particle counts were measured in real-time for House #3 (before and during intervention) using an optical particle counter (model 1.108, Grimm Technologies, Douglasville, GA). This instrument measures the number concentration of particles in 15 size ranges from >0.3 to 20.0μm. An averaging time of 1 min was used in this study.

Respirator efficiency testing was performed by measuring Workplace Protection Factor (WPF) against fungal spores collected in House #3. The Institutional Review Board of The University of Cincinnati

approved this sampling protocol and informed written consent was obtained by the university investigator wearing the respirators. The same subject wore two types of respirators: a disposable N-95 filtering facepiece respirator (8110, 3M, St. Paul, Minnesota) and an elastomeric half-facepiece respirator (North 5500-30 with North filter cartridge gas & vapor with P100 particulate filter, North Safety Products, Cranston, Rhode Island). The experimental protocols and the type of the N-95 filtering facepiece respirator were the same as those described in an agriculture study (Lee et al. 2005). In brief, the subject was fit-tested before the experiment using the Portacount method. The subject wore the test respirator, which was connected to a newly developed personal sampling set-up for 30 min. In the sampling set-up, fungal spores were collected from inside and outside the respirator onto polycarbonate filters. Concentration of fungal spores (spores/m^3) was determined through microscopic counting of the filters (Adhikari et al. 2003; Lee, S-A et al. 2006). WPF was calculated by dividing the fungal spore concentration inside the respirator by that outside the respirator near the subject's breathing zone. The test was repeated twice with the N-95 respirator and 4 times with the elastomeric respirator.

Outdoor samples: The battery-operated pumps were placed 3 m outside the front door for collection of one pre-intervention sample outside of House #3, one sample during the intervention for House #2, and post-intervention samples for all three homes. The high flow pumps were placed at 3 and 10 m outside the front door and were used in collection of samples before and during intervention. Lack of electricity precluded post-intervention sampling in Houses #2 and #3. Also, some outdoor samples were not collected during inclement weather due to concerns regarding technician safety and equipment damage.

Field blanks: On sampling days when intervention occurred, we collected a blank BioCell in order to assess fungal spores that could have been on the collection media before sampling, that were introduced in the moments before starting and after stopping the pump, and during sample transport. The BioCell

cassette was opened for 1 minute in the house (without attachment to a sampling pump), then sealed. All blanks were negative for fungal spores.

Analytical methods

Culture of fungi: Teflon filters were placed in 5 ml pyrogen-free water with 0.05% Tween 20 in sterile plastic tubes and shaken for 1 hour at 25 ^{o}C. Samples were vortexed and 100 µl aliquots (undiluted, 1:10, and 1:100 dilution) were spread-plated onto two types of culture media, dichloran glycerol (DG-18) and malt extract agar (MEA). Culture plates were incubated at two different temperatures, 25^{o}C (7-10 days) and 37 ^{o}C (2-3 days). For each type of media and each temperature, the plates with nearest 10-30 colonies were counted. Culturable fungi were reported in colony-forming units (CFU).

Fungal spore counts: Fungal spores were analyzed by direct microscopic examination of 27% of the impaction surface, using transverse scans across the spore deposit at 400X magnification. Larger, infrequently recovered spore types were counted by scanning the full impaction surface at 200X magnification. In very high spore concentration situations, where resulting spore densities were extreme, fewer transverse scans were performed using a 120 µm reticule (for example, 0.017% of the deposit was scanned in one sample). Most spores were identified to genus; *Aspergillus, Penicillium, Eurotium*, and *Paeciloymyces* are difficult to discern by this method and were grouped into one category.

PCR: Aliquots of the Teflon filter extracts were analyzed by P & K Microbiological Services, Inc (Cherry Hill, NJ) for species-specific quantification using quantitative Polymerase Chain Reaction (qPCR) following the methods previously described (Haugland et al. 2004, Vesper et al. 2004). Nucleic acids were extracted using the bead-beating technique. Reference controls were used as positive quantitative controls. Independent qPCR analyses were performed using primers and probes validated specifically for the 23 species/species groups of interest. The 23 taxa included: *Acremonium strictum,*

Alternaria alternata, Aspergillus flavus/oryzae, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus sydowii, Aspergillus ustus, Aspergillus versicolor, Eurotium (Aspergillus) amstelodami, Chaetomium globosum, Cladosporium cladosporioides, Memnoniella echinata, Paecilomyces variotii, Penicillium aurantiogriseum, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium purpurogenum, Penicillium variabile, Scopulariopsis brevicaulis/fusca, Stachybotrys chartarum, Trichoderma viride/koningii, and *Ulocladium botrytis.*

Endotoxin: Aliquots of the Teflon filter extracts were analyzed for endotoxin by the kinetic chromogenic *Limulus* Amebocyte Lysate assay (Thorne 2000; Vojta et al. 2002). Levels of endotoxin were reported in endotoxin units (EU).

Results

Comparison of levels before during and after intervention

The baseline levels of mold and endotoxin, some of which varied by orders of magnitude within homes, are shown in Table 3. The pre-intervention samples for House #1 were taken after some belongings had been removed the prior day, yet the baseline levels were still generally below those of other two houses. What is clear from the graphs in Figure 1 is that post intervention levels of all bioaerosols in each house tended to be lower than at pre-intervention, except endotoxin in House #2 which was moderately elevated and culturable mold in House #1 which had post-intervention levels similar to those collected pre-intervention. Because generators were not available at all sampling periods at Houses #2 and #3, several mold spore count measurements could not be collected. However, House #1 had spore measurements from all three timepoints, and the pattern was similar to that of the culturable fungi, PCR, and endotoxin results; levels increased during work and then decreased post-intervention (Figure 2).

Differences/ similarities in fungal taxa profiles

Interpretations of the predominant fungi recovered in the air samples differ depending upon the type of analysis. In Figure 3, *Aspergillus, Paecilomyces*, and *Penicillium* were among the most frequently detected fungal taxa as determined by all three methodologies (culture, PCR, and spore counting). Most of the spore count was due to *Aspergillus/Pencillium* spores. In House #1, *Stachybotrys* was recovered in the living room and outdoor air during the intervention but decreased to non-detectable levels by the post-intervention sampling visit. *Cladosporium* was not frequently recovered from culture; however, it was detected by PCR and spore counts frequently, but at low concentrations relative to *Aspergillus* and *Penicillium*. In addition, spores of *Curvularia* and the Basidiomycota (including rusts, smuts, and basidiospores) were identified by microscopy. Neither PCR nor culture methods used in this analysis had the ability to measure spores of the Basidiomycota. Culture at 25^{o}C and spore counting revealed that *Trichoderma* was commonly recovered in the indoor air samples. A PCR probe for *Trichoderma virde/koningii* had a low recovery suggesting the other methods may have been detecting another common *Trichoderma* species such as *T. harzianum*.

Particle counts

Figure 4 presents results on the total particle counts and Figure 5 presents the size-selective particle concentrations. For the data analysis, the 15 channels of the optical particle counter were combined into four size ranges: 0.3-0.8, 0.8-3.0, 3.0-7.5, and 7.5-20.0 μm. Results show that number concentrations of particles of all sizes increased during intervention. Most clearly this was seen for particles >0.8 μm. On the other hand, during lunch break, the concentration of particles >0.8 μm decreased almost to the same level as was measured before the intervention.

Efficiency of Respiratory Protection

Table 4 presents the WPF values for the two types of tested respirators. The average WPF against fungal spores for the elastomeric respirator was higher than that measured for the N-95 filtering facepiece respirator.

Discussion

A critical finding of this study is that in homes that held flood waters for several weeks, our flood cleanup techniques were associated with a reduction in mold and endotoxin levels. In all three homes, the interventions decreased mold levels and in some cases, the decreases were several orders of magnitude. In fact, the only measure that increased from pre- to post intervention was endotoxin in House #2. This house had the most extensive flood damage, and during the intervention, endotoxin was higher compared with the other houses. As endotoxin is cell wall component of Gram negative bacteria, we speculate that the bacteria were killed, but that endotoxin remained in the settled dust after the intervention. Nonetheless, the levels of mold (as determined by three different methodologies) were drastically reduced after the intervention in this house.

A strength of our study was the multi-pronged assessment of mold exposure. From our data, it is clear that the use of any one of the analytical methodologies (culture, microscopy, and PCR) would have shown that baseline levels of mold were high compared to agricultural, industrial, and other home environments. However, each analytical method gave different insight that will hopefully inform future investigations in New Orleans homes and other environments. In contrast to culture methods to detect and enumerate fungi, spore microscopy, and qPCR do not require viable fungal elements.. In addition, spore microscopy and qPCR detect not only dead fungi but those that compete poorly on the various media used to grow fungi. The culture-based analyses (on both MEA and DG18 agars) can underestimate the populations of these some *Aspergillus* species by orders of magnitude when compared with qPCR (Meklin et al. 2004). On the other hand, Basidiomycota spores (the common mushroom is in this fungal

phylum) generally do not grow well on culture plates and a probe was not available for PCR analysis, therefore, only spore microscopy enabled detection of this potentially allergenic group of mold (Horner et al. 1995; Lehrer et al. 1986). The low frequency of *Trichoderma* and *Alternaria* detection by PCR compared to the moderate to high frequency of recovery by direct microscopy and, to a lesser extent, culture, likely reflects the airborne presence of taxa (e.g., *Trichoderma harzianum* and *Alternaria tenuissima*) for which PCR probes were not used or available. The spore microscopy also revealed that *Curvularia* was common in the houses. Although a PCR probe for *Curvularia* was not available for analysis of these samples, culture analysis should have been able to grow *Curvularia.* The low prevalence of culturable *Cladosporium* contrasts with the high prevalence, as determined by microscopy and PCR. *Cladosporium* is one of the most common fungal taxa recovered indoors and outdoors throughout the world (Chew et al. 2001; Chew et al. 2003, Institute of Medicine 2004; Levetin 1995; Su et al. 2001). *Cladosporium* competes well with many of the other taxa that we recovered, so the reason for its decreased culturability in our samples remains elusive.

At baseline and particularly during intervention, we observed household levels of mold and endotoxin that equaled or surpassed those in wastewater treatment plants, cotton mills, and agricultural environments (Christiani et al. 1993; Lee, J et al. 2006; Lee, S-A et al. 2006; Spaan et al. 2006). Interestingly, the outdoor measurements were also higher than expected ranging from 10 to 25 EU/m^3. These exceed the average outdoor endotoxin measurements from 13 southern California communities of 0.44 EU/m^3 (Mueller-Anneling et al. 2004). In addition, the baseline levels of culturable mold in the three houses averaged 352,701 CFU/m^3 which was much higher than the average (2,190 CFU/m^3) or maximum level (48,760 CFU/m^3) in homes sampled after the 1993 Mississippi River flood (Ross et al. 2000). The comparison with the Mississippi River flood study may somewhat overstate the disparity between the two floods since sampling in the prior study occurred one year after the flood and only 40% of the residents in that study had reported flood damage in their homes. Nonetheless, the levels of mold in these homes were extremely high. Given the level of visible mold observed when we first entered our

demonstration homes, we were concerned about the level of respiratory protection required for entry and clean-up, and our measurements of both the mold levels and the respirator WPFs supported our concern.

By the time we planned the initial visit to House #3, we were able to characterize the particle sizes and conduct experiments to test the WPF of a disposable N-95 respirator and an elastomeric half-facepiece respirator. Our data indicate that although counts of larger particles decreased drastically during lunch, taking almost 30 min to reach the lowest point, and reached counts of 50, 500, and 5,000 per liter of air (i.e., 50,000, 500,000, 5,000,000 particles/m^3) depending upon the size range. These data, combined with the measurements of mold spores, culturable mold, and endotoxin suggest that even during periods of inactivity, a substantial portion of the particle counts could be comprised of fungal and bacterial material.

The choice of respirator depends on the expected level of contamination. Currently, no threshold limit values for mold or endotoxin in the United States; however, a Dutch occupational health standard for endotoxin (50 EU/m3) existed for a brief time (Douwes et al. 2003). Also, excessive mold can be cited as a health concern by the U.S. Occupational Safety and Health Administration (U.S. OSHA) under their general duty clause. Still, no defined level of mold is listed that warrants a specific level of respiratory protection. An Assigned Protection Factor (APF) can be used in the initial selection of the respirator type. It gives the level of the respiratory protection that a properly functioning respirator or class of respirators would be expected to provide to properly fitted and trained users in the workplace. The APF for both filtering facepiece and elastomeric half-facepiece respirators is 10 (e.g., 5,000,000 particles/m^3 would be reduced to 500,000 particles/m^3) (U.S. OSHA 2003). We found that that in the field, the WPF was lower for the N95 respirator. In a previous study, the WPF against fungal spores was measured in agricultural environments (Lee et al. 2005), and the geometric mean (GM) and geometric standard deviation (GSD) of 21 WPF data points was 25 and 9.9, respectively. In addition, the WPF was found to

decrease with decreasing spore size. The values obtained from the N-95 respirator in House #3 were somewhat lower than those in agricultural environments. This could be due the composition of fungal load in the moldy building where small *Aspergillus/Penicillium* spores predominated. On the other hand, the WPF for the elastomeric respirator was clearly higher than the values obtained for N-95 respirator in this study or in the previous study in agriculture. Although the number of WPF data points in this study is low, the results suggest that the elastomeric half-facepiece respirators offer at least 10 times protection against fungal spores. As the fungal spore concentrations were extremely high during the renovation, it is questionable if the protection offered by N95 filtering facepiece or even with the elastomeric respirators is sufficient. Furthermore, our WPF-values are based on microscopic counting of intact spores. Recent studies have shown that exposure to fungi occurs also through submicrometer fungal fragments (Foto et al. 2005; Gorny et al. 2002; Green et al. 2005). These particles may penetrate at even higher rates as intact spores because the filter materials commonly used in N95 respirators have maximum particle penetration around 0.03 – 0.07 μm (Balazy et al. 2006).

The post-intervention findings in House #1 highlight the critical importance of fully cleaning and drying a home. The upper walls and ceilings in the house were not vacuumed as part of the initial cleanup procedures. In addition, a small water leak saturated a portion of the concrete floor after work was completed. Because possessions remained inside, the house was closed and the humidity levels could have created a climate hospitable for further mold growth. The fact that culturable mold levels in this home were not substantially lower after intervention than before is likely related to these factors. After post-intervention sampling was completed, the water leak in House #1 was fixed and cleaning and mechanical drying was conducted.

House #1 also offers a cautionary note about the risks involved with leaving some of the original drywall in a home. Some flood cleanup guidance suggests in homes with minimal flooding, removing drywall on

walls to 1.2 m (4 feet is the width of a standard sheet of drywall) instead of to the ceiling can save thousands of dollars in restoration costs (ARC and FEMA 1992). However, many homes were submerged for weeks after Hurricane Katrina and although the water may have only wicked from the water line to the first 1.2 m, the homes were usually closed and the summer heat resulted in humidity levels similar to that of a terrarium. In our study, the house with the lowest water line had visible mold growth in the wall cavities above 1.2 m after treatment. While we cannot be certain that the growth would have occurred had cleaning and drying been adequate, the potential health risks of leaving the original drywall in the home must be taken into consideration.

The question of whether household bleach is an effective treatment mechanism was one of the most debated topics among the advisory group. While it can have adverse environmental health effects, a dilute solution of bleach was used due to widespread concern of bacterial contamination, evidence that it could denature allergens (Chen and Eggleston 2001; Matsui et al. 2003), and possibly inactivate mycotoxins (Wilson et al. 2004). Bleach was selected primarily based on the federal guidance and because it was widely accessible. In House #2, bleach was applied to the wooden building members while in House #3 it was not. Because the post-intervention mold findings were similar in the two homes, we are encouraged that intensive dry cleaning followed by the application of borates appears to control mold growth. The use of dry cleaning without wet cleaning the wood had the added benefit of reducing the time of flood cleanup because the workers did not need to allow the wood to dry prior to applying borates. We are aware that research is under that examines alternatives to bleach. If effective alternatives are identified, we would encourage their use to be incorporated into the federal emergency response protocols. Ideally the products should be accessible to consumers (both available and inexpensive) to enable their adoption.

There are several noteworthy limitations to this demonstration, including: 1) the small sample size; 2) inconsistency of number of samples collected; and 3) possible lack of generalizability to other homes

because of home selection process (Hung et al. 2005). The number of homes was necessarily small so that we could quickly try different types of clean-up procedures and assess their feasibility and efficacy. To conduct the interventions in a larger set of homes would have prevented expeditious reporting of findings. We had an inconsistent number of samples due to lack of electricity (as of July 22, 2006, this was still a problem for much of New Orleans (Nossiter 2006) and lack of access to a fully functioning laboratory in New Orleans. We selected houses with a range of flood damage; however, the houses were typical New Orleans building structures, and the level of flooding was typical of many homes in the affected areas of New Orleans. The homes in Mississippi that were directly in the path of Hurricane Katrina, sustained heavy wind damage, and we do not believe that our intervention results can be generalized to those homes. Nonetheless, our discussion of respiratory protection should be applicable to those homes with extensive mold growth.

The main goals for this pilot project were to synthesize, field test, and evaluate existing flood clean-up methods. For all houses, we removed 1.2 m or more of sheetrock, conducted HEPA vacuuming, used a borate salt solution to help prevent mold growth, and used bleach as a disinfectant. Using a variety of sampling and analytical methods, we observed airborne levels of mold and endotoxin that often increased orders of magnitude during the intervention and determined that workplace protection factors of some respirators can be suboptimal in such conditions. While the generally accepted mold remediation protocols reduced bioaerosols in the demonstration houses, myriad issues including the qualifications of those performing the work (including homeowners), depth and duration of flooding, and the availability of electricity and supplies can impact the feasibility and ultimately the success of flood clean-up efforts. Our pilot project was not designed for determining whether the demonstration homes were safe for re-occupancy. Rather, the demonstration examined the extent to which homes that experienced significant and prolonged exposure to flood waters could be satisfactorily cleaned in order to enable reconstruction.

Future research may include revisiting these homes following reconstruction to determine whether the low bioaerosol levels persisted or even continued to decline.

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Table 1. Baseline conditions and demonstration activities in houses.

House ID	Baseline Description	Removal of flood-damaged items	Cleaning	Drying	Application of Biostatic Agent
House #1	- Built in 1987 - Two-story, slab on grade with attached apartment on the first floor. - Water line at 0.3m above floor - Roof had been patched with a temporary tarp before initiating flood clean-up - Electricity operational	- Owner moved some cleaned personal belongings (clothes, items in cardboard boxes) to the second floor of the home for storage. - Bottom 1.2m of drywall was removed	- Durable furnishings were wet-cleaned, wiped dry and placed in storage inside the home. - Only concrete and vinyl floor and countertops were wet-cleaned - Ceiling was not dry-cleaned	- Owner left an upstairs ceiling fan on throughout the duration of the demonstration project, but windows were closed	- Bora-Care (1:3 dilution)
House #2	- Approximately 100 years old - One-story raised home with plywood underlayment on 0.6m piers - Water line at 1.8m above floor - Electricity not operational	- Furnishings and appliances were too damaged to salvage, so workers removed all furnishings for disposal. - Floor to ceiling drywall was removed	- All surfaces were wet- and dry-cleaned	- Windows left open for two weeks before applying a biostat treatment - Windows also left open after application of biostatic agent	- Bora-Care (1:4 dilution) in front half of home - Termite Prufe in back half of home
House #3	- Built in 2004 - One-story raised home with plywood underlayment on 0.9m piers - Water line at 0.6m above floor - Electricity not operational	- Owners had removed all personal belongings, furniture, appliances and lower kitchen cabinets prior to the demonstration at Home #3. - Floor to ceiling drywall was removed	- All surfaces were dry-cleaned - Only toilets, sinks and bathtubs and vinyl floors were wet-cleaned	- Windows left open after application of biostatic agent	- Termite Prufe

Table 2. Air sampling activities conducted (indicated by X) for houses.

Activity	House #1	House #2	House #3
Indoor Air (culturable fungi, endotoxin, PCR)			
Pre-work	X	X	X
During work	X	X	X
Post-work	X	X	X
Indoor Air (fungal spores)			
Pre-work	X	X	X
During work	X	X	X
Post-work	X		
Outdoor Air (culturable fungi, endotoxin, PCR)			
Pre-work			X
During work		X	
Post-work	X	X	X
Outdoor Air (fungal spores)			
Pre-work	X	X	X
During work	X	X	X
Post-work	X		
Respirator efficiency testing			X

Table 3. Baseline indoor concentrations of mold and endotoxin

	Total Culturable Mold (cfu/m^3) [a]	Total Mold Spore Counts ($Spores/m^3$)	PCR results (Spore equivalents/m^3)	Endotoxin (EU/m^3)
House #1	22,000-46,000	82,381	80,779	43
House #2	268,000-515,000	202,634	1,039,841	17
House #3	Bedroom= 29,000-59,000 LR/Kitchen=332,000-342,000	634,651	Bedroom=77,911 LR/Kitchen=178,067	Bedroom=100 LR/Kitchen=139

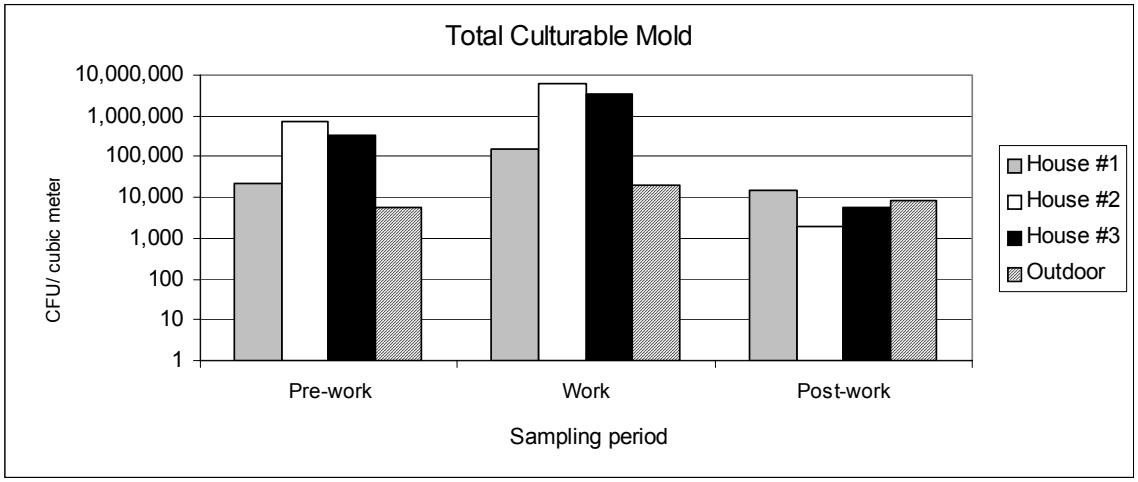
[a] The average of samples grown on two different media and incubated at two different temperatures (resulting in n=4 plates per sample collected).

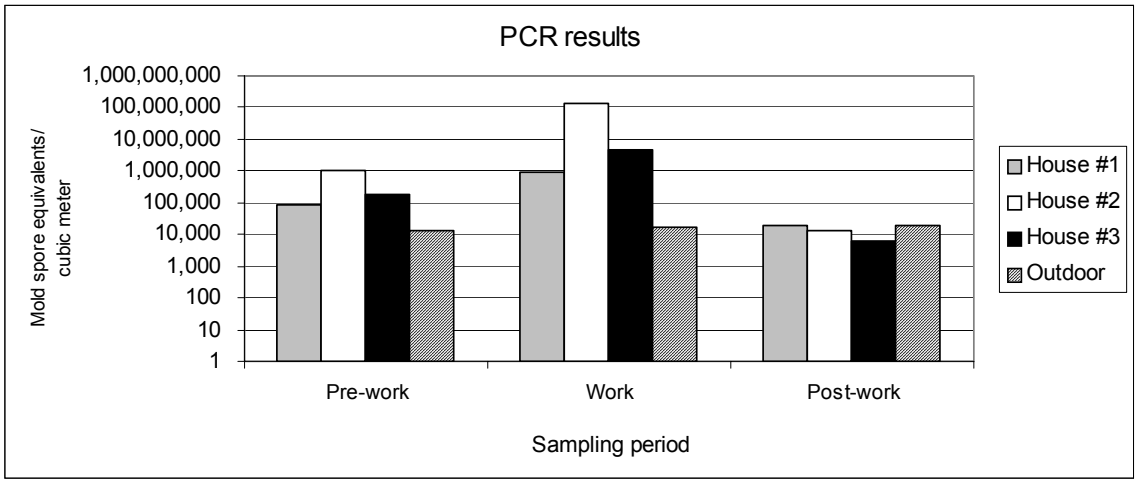
Table 4. WPF against fungal spores with two types of respirators

Respirator type	Number of Subjects	Number of WPFs	WPF against fungal spores: GM(GSD)
N-95 filtering facepiece	1	2	5 (3.6)
Elastometric half-facepiece	1	4	40 (2.9)

Figure Legends

1. Figure 1. Mold and endotoxin results. Filter samples from the three houses were extracted and aliquoted for three different analytical methods (Total culturable fungi (results only shown for those grown on MEA and cultured at 25^{o}C), PCR, and endotoxin). Home #1 "work" is average of 2 measurements (upstairs and downstairs). Outdoor "pre-work" is only represented by home #3. Outdoor "work" is only represented by Home #2. Outdoor post-work is average of 3 measurements (Home1, Home 2, and Home 3).
2. Figure 2. Mold spore count results. Only House #1 had a post-work measurement inside the house. Average of 3 measurements (House 1, House 2, and House 3) was used for outdoor pre-work and work levels. House #1 spore counts decreased 77.6%.
3. Figure 3. Frequency of Fungal Taxa. Samples from indoor air and outdoor air of all three houses and during different timepoints were grouped together. Sample size limited interpretation when stratifying by location, and timepoints. Culture at 25^{o}C (n=35), Culture at 37^{o}C (n=35), Spore counting (n=17), and PCR (n=21)
4. Figure 4. Total particle concentrations before and during the renovation.
5. Figure 5. Size-selective particle concentrations.


Total Culturable Mold
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
CFU/ cubic meter
Pre-work
Work
Post-work
Sampling period
House #1
House #2
House #3
Outdoor


PCR results
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
Mold spore equivalents/ cubic meter
Pre-work
Work
Post-work
Sampling period
House #1
House #2
House #3
Outdoor

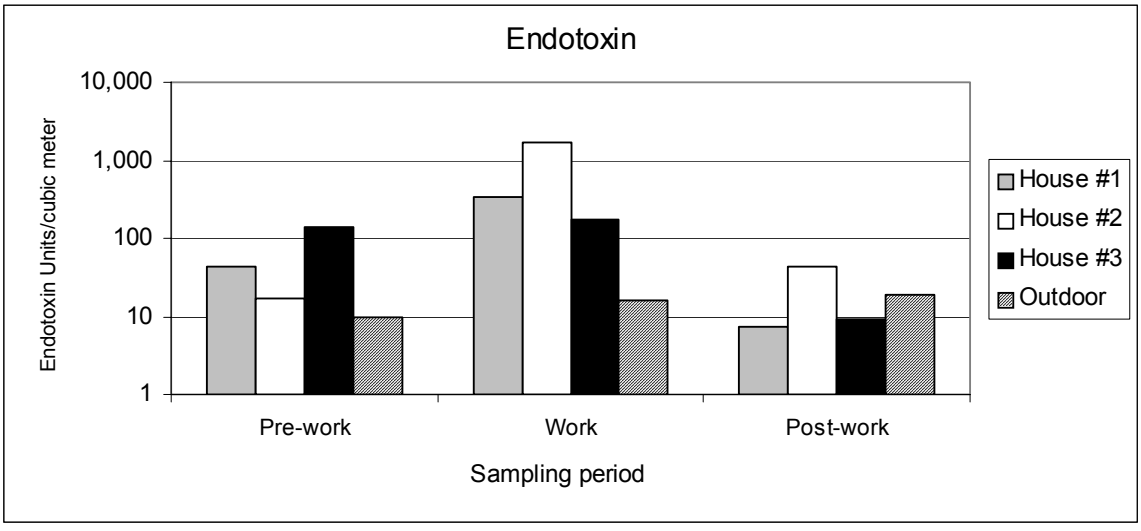

Endotoxin
10,000
1,000
100
10
1
Endotoxin Units/cubic meter
Pre-work
Work
Post-work
Sampling period
House #1
House #2
House #3
Outdoor

Figure 1.

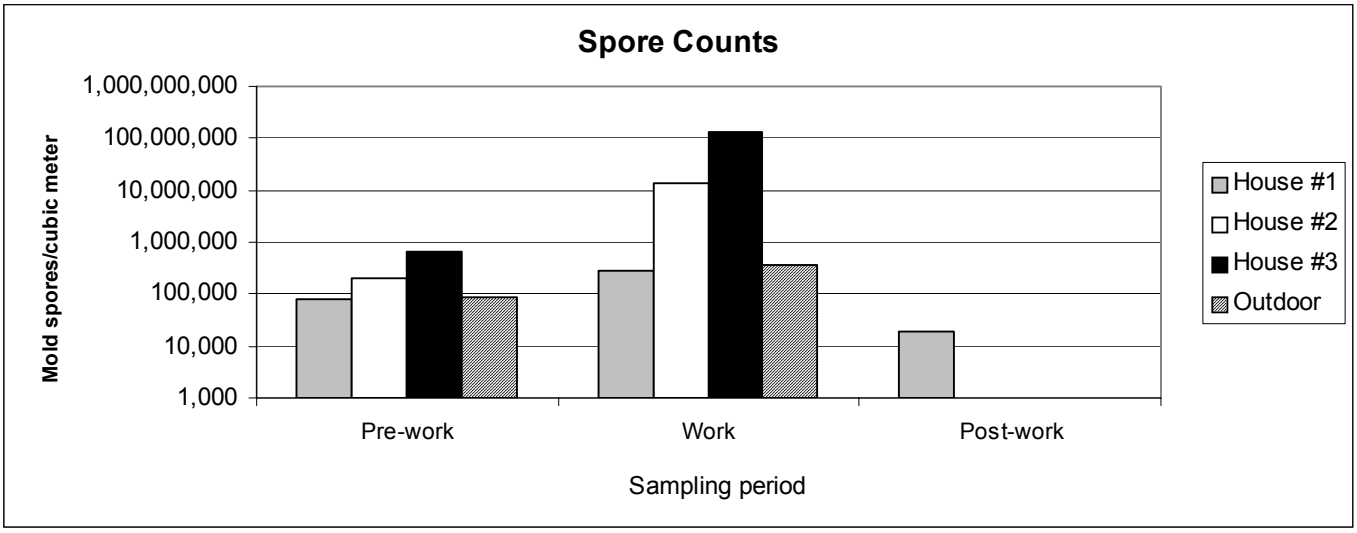

Spore Counts
Mold spores/cubic meter
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
Pre-work
Work
Post-work
Sampling period
House #1
House #2
House #3
Outdoor

Figure 2.

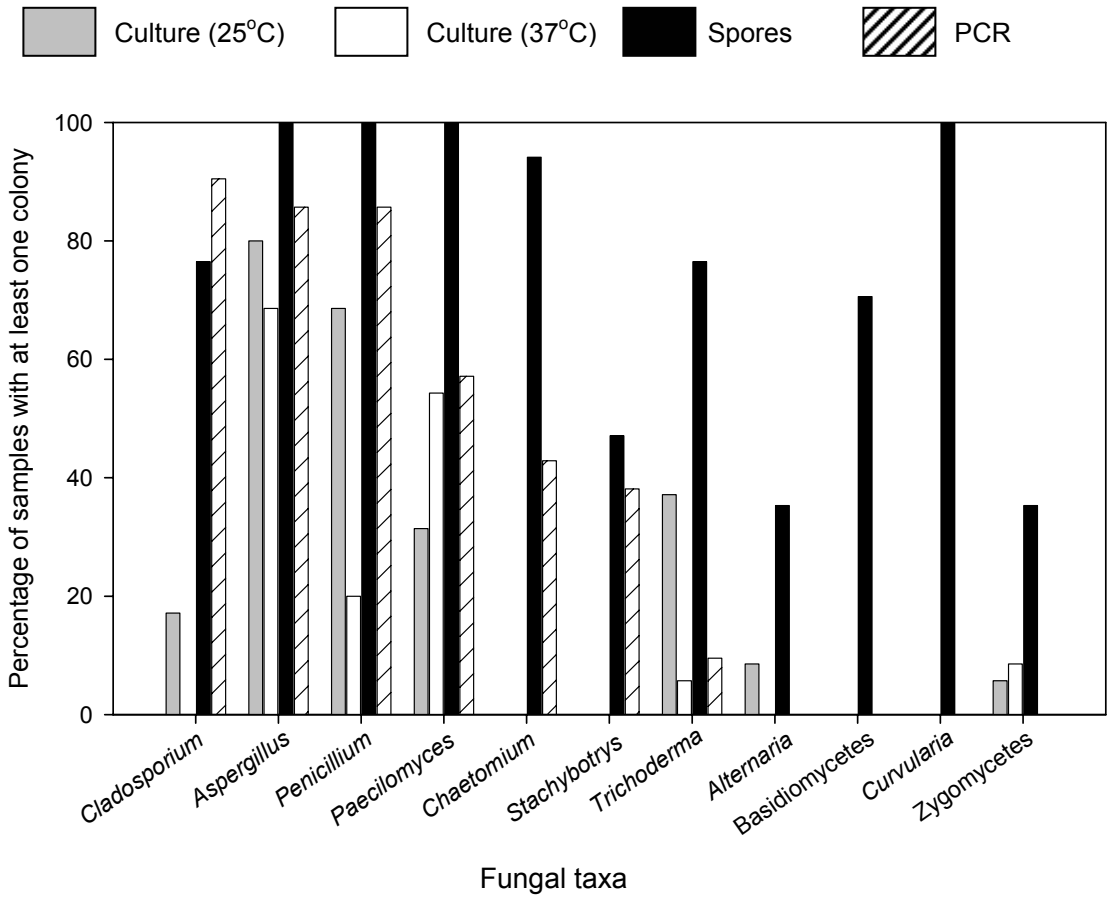

Culture (25°C)
Culture (37°C)
Spores
PCR
Percentage of samples with at least one colony
100
80
60
40
20
0
Cladosporium
Aspergillus
Penicillium
Paecilomyces
Chaetomium
Stachybotrys
Trichoderma
Alternaria
Basidiomycetes
Curvularia
Zygomycetes
Fungal taxa

Figure 3.

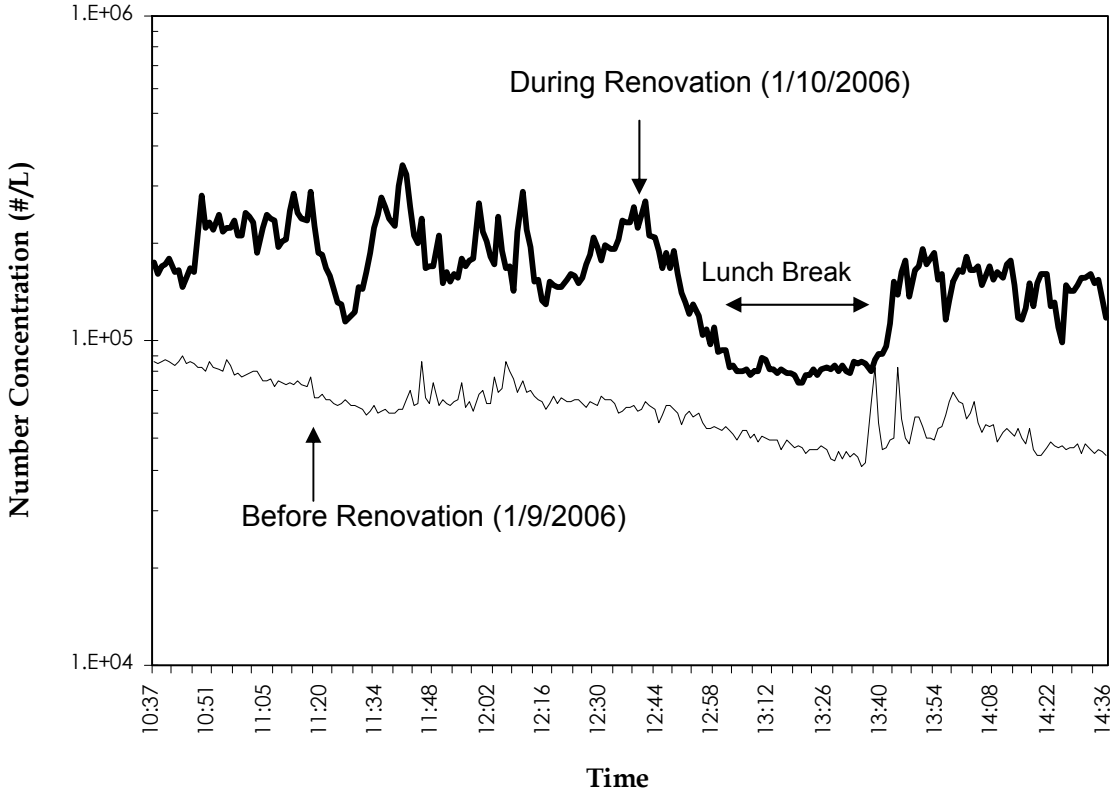

1.E+06
1.E+05
1.E+04
Number Concentration (#/L)
During Renovation (1/10/2006)
Lunch Break
Before Renovation (1/9/2006)
10:37
10:51
11:05
11:20
11:34
11:48
12:02
12:16
12:30
12:44
12:58
13:12
13:26
13:40
13:54
14:08
14:22
14:36
Time

Figure 4.

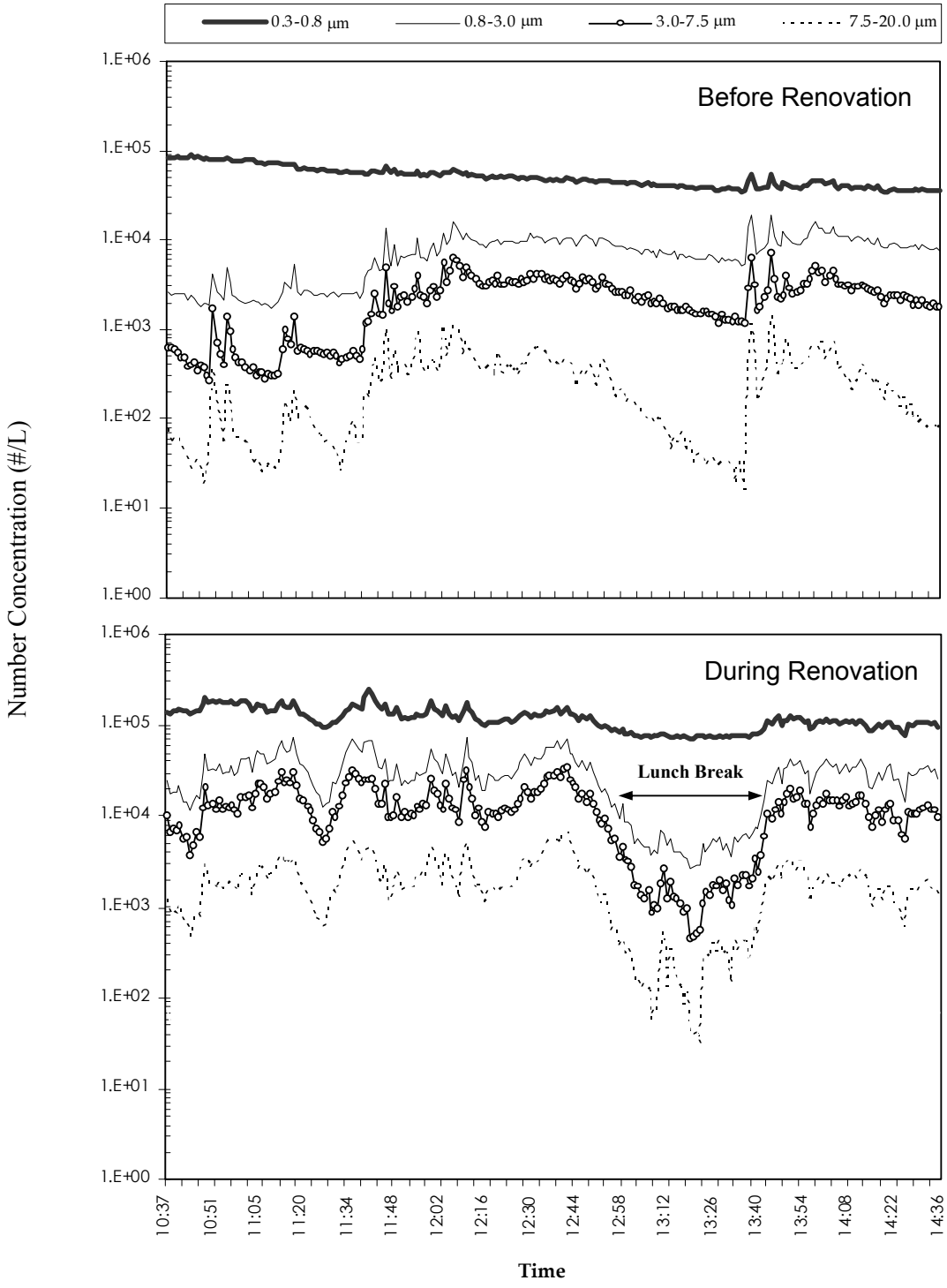

0.3-0.8 μm
0.8-3.0 μm
3.0-7.5 μm
7.5-20.0 μm
Before Renovation
During Renovation
Lunch Break
Number Concentration (#/L)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
10:37
10:51
11:05
11:20
11:34
11:48
12:02
12:16
12:30
12:44
12:58
13:12
13:26
13:40
13:54
14:08
14:22
14:36
Time

Figure 5.